Michigan Department of Community Health
MDCH
John Engler, Governor
James K. Haveman, Jr., Director

LabLink

Michigan Department of Community Health
Bureau of Laboratories

Vol. 7 No. 3 Spring 2002

Bug Bytes: Survey Results from Michigan Clinical Laboratories on the Culture Protocols for O157:H7

John Dyke, Ph.D.
Bureau of Laboratories

Foodborne illnesses continue to be a significant public health concern. Of the agents associated with foodborne illness, *Escherichia coli* O157:H7 and other shiga toxin producing strains continue to be the most frequently involved agent. This was the stimulus that brought the question as to what the various laboratories within the state were doing with respect to the recognition of *Escherichia coli* O157:H7 in stool specimens and what was the current status of laboratories to detect shiga toxin producing strains.

A questionnaire was developed to focus on whether laboratories, which are licensed to do microbiology, provide services for the recovery of *Escherichia coli* O157:H7 including how requests for testing were initiated, what methods were employed to recover *Escherichia coli* O157:H7 from clinical specimens and what methods were used to confirm its presence. Lastly, was an isolate sent to the Michigan Department of Community Health (MDCH) laboratories.

The survey was sent to 123 clinical laboratories by a previously established fax network. Of the 123 questionnaires sent, 102 were returned. The following are the results derived from the respondents.

On the question as to whether services were provided on site, 24 laboratories (23%) did not currently have on site services. All 24 laboratories did forward requests for culture of *Escherichia coli* O157:H7 to a reference laboratory. The remaining 78 laboratories (77%) had methods in place for the recovery of *Escherichia coli* O157:H7.

For laboratories that had on site services, protocols for examining clinical specimens for *Escherichia coli* O157:H7 were varied. Five laboratories (6%) culture specimens only when ordered by the physician. There were 22 laboratories (28%) that cultured specimens using the criteria of physician orders and/or on bloody stool specimens.

The majority of surveyed laboratories (59%) examine specimens for *Escherichia coli* O157:H7 as part of their routine stool culture procedure. Three laboratories (3%) culture all stools for *Escherichia coli* O157:H7 on children seven years of age or younger. The criteria for one hospital was to examine all bloody stools. Lastly, one reference laboratory provided services as requested.

Screening and confirmation methods also varied among the responding laboratories. The majority of laboratories (53%) that provided services for the recovery of *Escherichia coli* O157:H7, used a combination of sorbitol MacConkey selective agar and latex agglutination serology on sorbitol negative colonies. There were 11 laboratories (14%) which employed the sorbitol MacConkey selective agar and referred sorbitol negative isolates to a reference laboratory for confirmation. Four laboratories (5%) used a stool culture method that did not include a selective media and determined the presence of *Escherichia coli* O157:H7 by biochemical identification. All of these laboratories used an automated system and if an isolate was identified as *Escherichia coli* O157:H7 it was sent to a reference laboratory for confirmation. The remaining three laboratories (4%) used a shiga toxin assay for screening. They submit isolates that screen positive to a reference laboratory or do biochemicals prior to sending the isolate to a reference laboratory.

One concern, answered by this survey, was whether the MDCH laboratories would have an isolate of *Escherichia coli* O157:H7 in the event an investigation of a foodborne outbreak occurred. The majority of the laboratories surveyed included in their stool culture procedure to submit an isolate to the MDCH laboratories. Those that were not currently following this practice were contacted and agreed to change their current procedure. In summary, the current procedures for the processing of stool specimens in clinical laboratories for the recovery of *Escherichia coli* O157:H7 is based upon current recommended practices.

The Michigan Department of Community Health would like to thank all the laboratories for their participation in this survey as well as for the quality of work they are doing to protect the health of the citizens of Michigan.

Escherichia coli Toxin Testing

William Schneider RM(AAM)
Enteric/STD/Chromatography Unit

The microbiology laboratory has been examining *Escherichia coli* cultures for the ability to produce Shiga toxins one and two (STX1 and STX2) since January 1, 1997. These toxin producing organisms are strongly implicated with hemorrhagic colitis and hemolytic uremic syndrome (HUS), particularly in children. This manifestation may result in kidney malfunction and often death.

E. coli cultures submitted for serotyping are examined, by DNA probe, for the ability to produce STX1 and/or STX2. Those strains probe positive for STX1 or STX2 are serotyped. MDCH has antisera for O157:H7, O111 and O126 strains. Toxin probe positive cultures that cannot be serotyped with this antisera are sent to the Centers for Disease Control (CDC) for serotyping. MDCH reports the STX results of all *E. coli* strains in addition to the serotyping results. Cultures negative for STX1 and STX2 are reported "serotype unknown." The following charts contain results of STX testing over the last five years.

Serotypes Identified

Serotype	1997	1998	1999	2000	2001
O157:H7	113	74	112	111	95
O157:NM	5	4	6	2	3
O157:H45	0	0	0	0	1
O18:H7	0	0	0	0	1
O22:H8	0	0	0	0	1
O26:H11	1	0	1	1	0
O45:H2	0	0	0	2	0
O55:H7	0	1	0	0	0
O110:H28	0	0	0	0	1
O111:NM	0	0	1	0	0
O113:H21	0	0	0	0	1
O119:H25	0	0	1	0	0
O121:H19	0	0	0	1	0
O171:H2	1	0	0	0	0
O Und:H8	0	0	0	1	0
O Und:NM	0	0	0	0	1
O145:NM	0	0	0	0	1
Unknown	379	311	244	225	221
Total	500	390	365	343	327

NM=nonmotile
UND=undetermined

Toxin by Serotype

Jan. 1, 1997-Dec. 31, 2001

Serotype	STX1 and STX2 Pos.	STX1Pos STX2Neg.	STX1 Neg. STX2 Pos.	STX1 and STX2 Neg.
O157:H7	411	3	90	2
O157:NM	4	0	16	0
O157:H45	0	0	0	1
O18:H7	0	0	0	1
O22:H8	1	0	0	0
O26:H11	0	2	0	1
O45:H2	0	2	0	0
O55:H7	0	1	0	0
O110:H28	0	1	1	0
O111:NM	1	0	0	0
O113:H21	0	0	1	0
O119:H25	0	1	0	0
O121:H19	0	0	1	0
O171:H2	0	1	0	0
O Und:H8	0	1	0	0
O Und :NM	1	0	0	0
O145:NM	0	0	1	0
Unknown	0	0	0	1380
Total	417	12	110	1385

Note that a majority of O157 cultures produce STX toxins, but some do not. Conversely, several other serotypes have the ability to produce STX toxins but they are rarer than O157 strains. Some non-O157 cultures were able to utilize sorbitol. This adds a new problem to identifying strains causing this disease.

FUN FUNGI.....

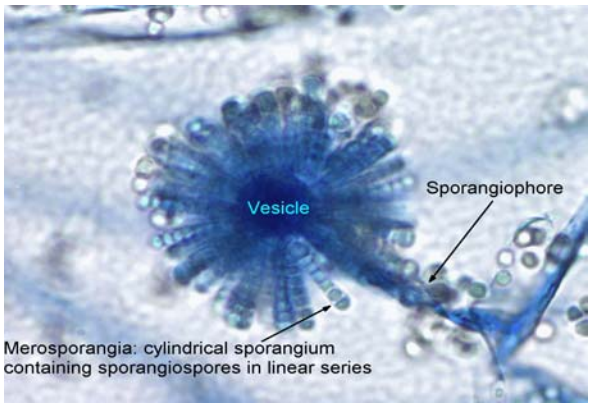
Syncephalastrum spp.

Sandy Arduin & Bruce Palma - Mycobacteriology/Mycology Unit

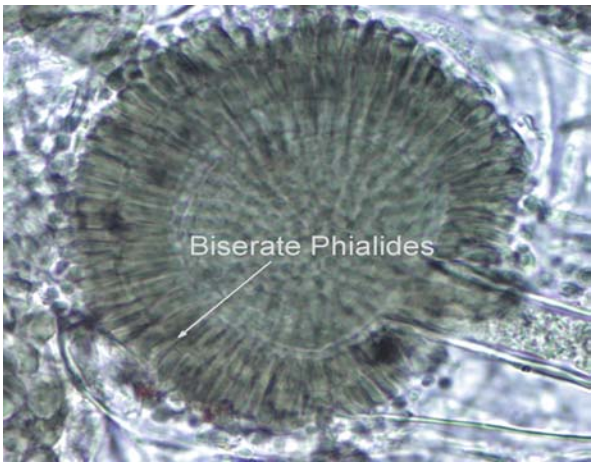
This Issue: *Syncephalastrum* spp.

Syncephalastrum spp. are classified as part of the order of Mucorales within the class of Zygomycetes. They are typically light to dark grey and wooly, with a pale reverse. The hyphae are broad with few or no septa. Sporangiophores (a specialized hypha giving rise to a sporangium) are branched, curved and terminate in a vesicle upon which finger-shaped merosporangia are fixed. Sporangiospores are round and form in a linear series within the interior of the merosporangia. Rhizoids are also present. *Syncephalastrum* spp. are saprophytic and rarely cause disease in humans. They are commonly isolated from animal dung or soil. Some microbiologists confuse this mould with *Aspergillus* spp. With careful microscopic observation, the merosporangia of *Syncephalastrum* spp. will not be confused with the phialides of *Aspergillus* spp.

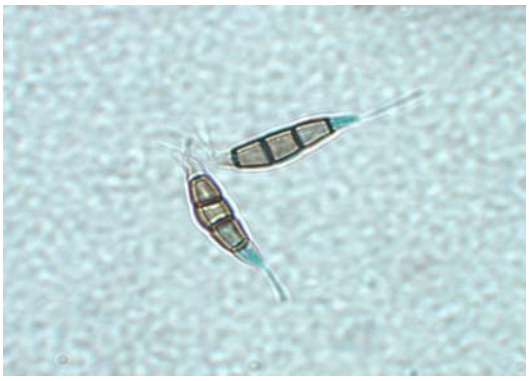
Syncephalastrum spp.



Aspergillus niger



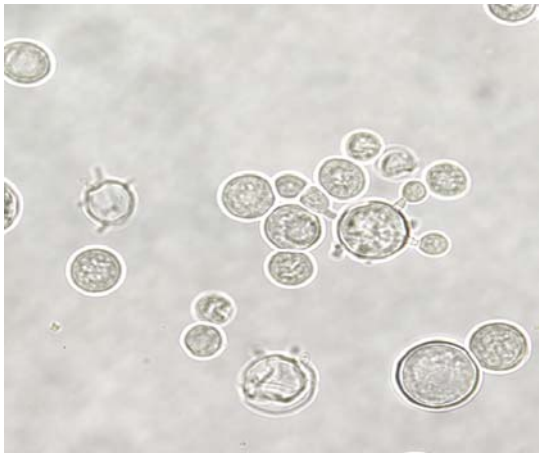
Last Issue's Picture Quiz Answer:



The mould in this photo is *Pestalotia* conidia. *Pestalotia* spp. are commonly found as parasites of plants and infrequently found in soil. *Pestalotia* spp. are characterized by the production of acervulus (a flat or saucer-shaped stroma or hyphal mat containing a stand of closely-packed condiophores) bearing dark phragmospores (multi-celled spores with transverse septa). These phragmospores have hyaline end cells each bearing two or more setulae (a delicate, hair-like, non-motile appendage found at the ends of the conidia).

This issue's picture quiz:

The isolate was received as an isolate from peritoneal fluid. Growth on Sabouraud's agar was tan, flat and wrinkled with a grey center. The mould formed a yeast phase when placed on cysteine agar at 37EC. This picture demonstrates the yeast phase.



What Mould Is This?

The Importance of Timely Specimen Collection and Transportation

Marilyn Boucher, C(ASCP),
Endocrine Unit

The newborn screening laboratory receives specimens mailed from all over Michigan. Several of the screened conditions are immediately life-threatening. MSUD (Maple Syrup Urine Disease), so-named for the maple odor emanating from an afflicted infant's wet diaper, is one such metabolic illness. Caused by the body's inability to metabolize the amino acid leucine, MSUD is treated with a restricted diet, special formula and vigilant medical care.

One of the criteria for inclusion in the screening program is a lack of early signs and symptoms. MSUD babies do not exhibit specific symptoms until it is too late to intervene.

If the birth is a normal, uncomplicated delivery, the baby goes home the next day and a newborn screen is collected, dried, sent to the laboratory and possibly to medical records unit, of the delivery hospital and then it is mailed to the MDCH newborn screening laboratory. Some hospitals use a courier system to deliver specimens.

At home, the new arrival may sleep excessively, even skip a feeding. Perhaps the behavior of the infant seems different from how he or she acted while in the hospital. There may be some prolonged or very shrill crying. If this is a first baby, the parents may not be able to differentiate normal crying from distressed crying. They may note a strangely familiar odor to the diapers but are not alarmed about it.

A phone call or visit to the pediatrician's office may be comforting to a new mom but, again, in the early stages of a genetic disease, there are no specific symptoms. The doctor may attribute a colicky, hypotonic, easily-startled infant to "new mother nerves". Mother and baby may be sent home with the time-honored advice: Relax.

Meanwhile, the newborn card may be left drying longer than necessary or marooned in the hospital's mailroom over a weekend. It may be mailed in an ordinary white office envelope instead of the state-provided yellow "clinical specimen" envelope with the proper post office box included. Many such small benign holdups can literally be the difference between a life or death situation.

Within 48 hours, a mother might arrive at her newborn's crib and find the baby convulsing spasmodically. Calling 911, if she's quick, will bring advanced life support to the scene. The infant may be sedated, intubated and given IV fluids. Hopefully the baby doesn't "crash" and have to be resuscitated with CPR. But these things can and do happen.

Once received into the MDCH laboratory, a newborn specimen is tested the same day. Strong positive values are called and faxed to the Pediatric Neurology Medical Clinic in Ann Arbor allowing for immediate contact of the family and treatment of the child. With timely specimen collection, a medical emergency, such as the one outlined above, can be averted. Greatly reduced morbidity is the outcome.

It is important to think of the familiar Newborn Screening card as a biological specimen requiring prompt attention and handling, not just another piece of outgoing mail.

From the Editor

It does not seem as if seven years have passed since the first *LabLink* went to print. Each year has been a learning experience. *LabLink* is designed as a communication tool between clinical laboratories, hospitals, county health departments and the state laboratories.

The goal of *LabLink* is twofold:

1) To provide the latest information on testing services available at MDCH.

2) To keep laboratorians and public health officials appraised of new and emerging issues.

This is a difficult task if the information we are providing does not reach the intended audience. It is critical to circulate the copy of *LabLink* that you receive at your institution. This way technologists working on the bench, communicable disease nurses and public health officers are kept up to date on the happenings at the state level. It is disheartening to appear at a meeting only to find that no one in attendance has ever seen the copy of the *LabLink* sent to their institution.

If you no longer wish to receive the *LabLink*, or know of someone who would like to receive it, simply fill out the form on the back page of this issue. We will try to honor all requests but due to budget restraints will have to limit the number of copies per institution. *LabLink* is available on the MDCH website by searching for "*LabLink*" at www.michigan.gov/mdch.

Our colleagues in hospital and community laboratories and regional health department laboratories are invited to submit items of interest to the *LabLink*. If you have an article to submit, or a topic that you wish to have discussed in future issues, please contact Susan Shiflett at (517) 335-9763 or at shifletts@michigan.gov.

DNA Amplification Testing The First Year

William Schneider RM(AAM)
Enteric/STD/Chromatography Unit

In January 2001, MDCH and the MDCH regional laboratories switched from DNA probes to a DNA amplification assay for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. Amplified tests offer increased sensitivity that should result in a significant increase in the number of infected patients identified, particularly with *Chlamydia*. In 2000, data from all the regional labs using DNA probes showed a 4.9 percent positivity rate for *Chlamydia* and a 2.8 percent rate for gonorrhea. Using the amplified testing during 2001, the rates were 7.0 percent for *Chlamydia* and 2.9 percent for gonorrhea. That is a 42.8 percent increase for *Chlamydia* and a more modest 3.6 percent increase for gonorrhea. The regional labs performed 123,054 tests in 2001. Compared to 2000, there was a 9.3 percent increase in the number of Chlamydia tests performed during 2001 and a 54.9 percent increase in the number of tests for gonorrhea. This large increase for gonorrhea is due to using a dual assay rather than individual tests and the fact that most of gonorrhea cultures have been replaced by the amplified test.

That is the good news. However, the new testing method has led to some new challenges as well as resurrecting ongoing problems.

Urine testing is available using this assay. However, urine specimens can only be tested for two days after collection if held at room temperature. Like swabs, they can be held for six days when refrigerated. Urine specimens do result in more indeterminate test results than swab specimens due to inhibitors excreted in urine. During 2001, there were indeterminate rates of 7.5 percent for urine specimens and a 0.4 percent swab specimens. There are very good reasons for urine testing (e.g., ease of collection) but the trade off is higher rates of indeterminate results which are more challenging to manage clinically.

In addition 1,715 specimens received in 2000 were unsatisfactory and not tested. Careful attention to detail would eliminate the vast majority of problem specimens.

Specimens older than six days accounted for 51.5 percent of all unsatisfactory reports. Specimens should, if possible, be sent out the same day they are collected. New security procedures and the transfer of specimens between agency mail delivery systems, the U.S. Postal Service and the State of Michigan mail delivery system adds some delay to delivery. If specimens are shipped promptly, these delays can be circumvented.

Leaking specimens, particularly urine samples. accounted for 18.5 percent of the unsatisfactory results. Initially, there was some difficulty finding a urine cup that did not leak and could still be packaged to meet federal shipping requirements. After testing several containers, a new one was selected and this problem dropped off dramatically. To decrease the number of leaking specimens, be sure to tighten the tops until the arrows on the container line up with the arrows on the lid. Leaking specimens will not be tested because it is difficult to clean up leaking specimens without introducing cross contamination with DNA. Amplification assays are very sensitive and any additional procedures which may cause cross contamination are avoided.

Specimen identification problems accounted for 17.4 percent of all problems. There was no name on the specimen for 14 percent and another 3.4 percent had names on the specimen that differed from the name on the test requisition. It is essential to have names or unique identifiers match exactly between the test requisition and the specimen. Data acquisition and specimen handling (DASH) personnel try to resolve these issues with submitters, but this is time consuming for both the agency and the laboratory and may result in additional delay in testing specimens.

A test requisition received without a specimen caused 4.9 percent of the unsatisfactory reports. This is self explanatory. This results in cost for mailing and handling at both ends for no reason.

These four other problems account for 92.3 percent of all unsatisfactory specimens. Other problems include incompatible specimen source, incompatible specimen collection kit, insufficient specimen volume and torn urine processing packets. All can be resolved by following the specimen collection guidelines distributed with the specimen collection kits.

Resolving these issues will improve patient care, decrease costs and save time, thus greatly improving the diagnosis and treatment of these disease agents.

The cost of this testing continues to climb. In an effort to help contain costs, the Sexually Transmitted Diseases and Family Planning Programs are asking submitters to carefully screen patients before testing to eliminate inappropriate testing. This will allow the limited funding to be used where it will accomplish the most in the elimination of these diseases.

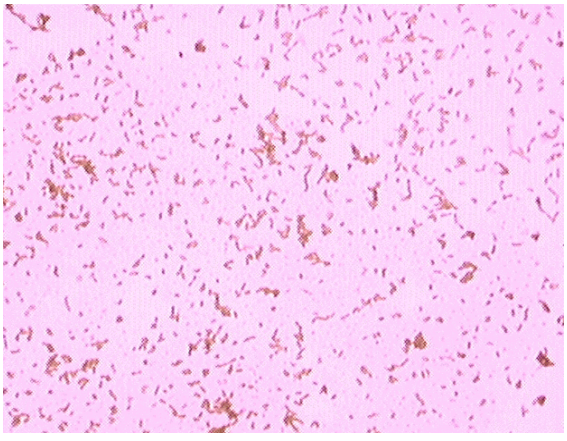
Quirky Bugs.....Odd Anaerobes

Thomas F. Edwards, BS, M(ASCP)
Reference Bacteriology

Occasionally, anaerobic gram negative bacilli are encountered that defy identification because they do not resemble any of the most commonly seen genera such as Bacteroides, Prevotella, Porphyromonas or Fusobacterium. Over the past year, the reference bacteriology unit at MDCH has received two such organisms as blood culture isolates.

The first was described only as an anaerobic gram negative rod isolated from an 80 year-old female. The organism was identified as *Desulfomonas pigra*. This organism is part of the normal colonic flora and is occasionally isolated from peritoneal fluid and abscesses.

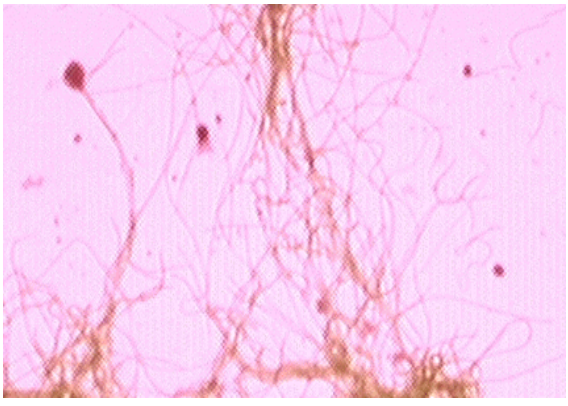
The organism is characterized as a gram-negative, straight rod with blunt rounded ends. On CDC blood agar, colonies are small, gray, semi-opaque, non-hemolytic, convex, circular and entire. This organism is nonmotile, non-spore forming, bile variable and negative for catalase, esculin, urease and indole. *D. pigra* is sensitive to kanamycin, but resistant to vancomycin and colistin. The organism is asaccharolytic and not found in the data base of most commercial identification systems. This organism can be presumptively identified by the production of acetic acid from chopped meat carbohydrate broth and the production of H_2S on a Triple Sugar Iron (TSI) slant.



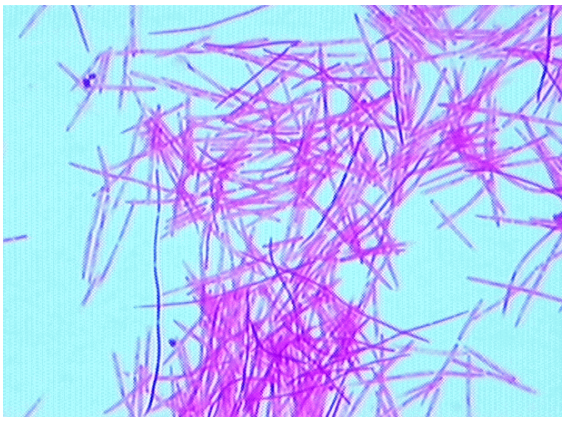
Desulfomonas pigra

The second organism was isolated from a 42 year-old male and was submitted as a micro-aerophilic, catalase and oxidase negative, gram-positive rod, possibly *Erysipelothrix*. The organism was identified as *Leptotrichia buccalis*. This organism is commonly found in the oral cavity, but may also occur in the intestinal tract and vagina. It can occasionally be isolated from the blood of immunocompromised individuals that have oral mucositis or other oral lesions.

L. buccalis is described as a slightly curved rod, 0.8-1.5 x 5-15 microns in size, with pointed or rounded ends, in pairs and chains. Cells are gram-negative, with gram-positive granules along the long axis, and can be totally gram-positive in young cultures. Colony morphology is smooth and colorless when young, and later will become convoluted (brain like) on the surface with a filamentous edge. The organism may grow slowly in an atmosphere containing five to ten percent CO_2. Although this organism is saccharolytic, it is not included in the data base of most commercial identification systems. It is esculin positive, but nitrate, urea, indole, catalase, and motility negative. Growth is not stimulated by 20 percent bile. The major end product of glucose metabolism is lactic acid. *Leptotrichia* spp. like *Fusobacterium* spp. are resistant to vancomycin but susceptible to kanamycin and colistin. While both fusobacteria and *Leptotrichia* cells are tapered, *Leptotrichia* are much larger than fusobacteria.



Fusobacterium nucleatum



Leptotrichia buccalis

References:

Holdeman, Moore, & Cato, *Anaerobic Laboratory Manual*, 4th ed., 1977, Blacksburg, VA, p 30-43.

Jousimiers-Somer, Summanen, & Finegold , "Anaerobic Gram-Negative Rods and Cocci," Murray et al, *Manual of Clinical Microbiology*, 7th ed., 1999, American Society for Microbiology, Washington D.C., pp 690-708.

Krieg et al, *Bergy's Manual of Systemic Microbiology*, Vol. 1, 1984, Baltimore, MD., pp 637-641, 672-3.

NOTICE
WEB ADDRESS CHANGE

The Bureau of Laboratories web page has recently moved to the Michigan.gov web site.

Our old site address will direct you to www.michigan.gov/mdch. From there, enter "Lab Services" into the Search box and press GO. The BOL site should be the first on the generated list. Please bookmark the new site so you don't have to go through the search process each time you visit.

Bureau Comings and Goings

The Bureau of Laboratories would like to welcome the following new employees. Beth Holben has joined the microbiology section as a microbiologist. Holben comes to the department from Sparrow Hospital in Lansing. Kristine Smith has joined the virology section as a microbiologist. Smith comes to MDCH from Ingham Regional Medical Center also in Lansing. Margo Ross as joined the mycobacteriology/mycology unit as a laboratory technician. Ross comes to MDCH from the Department of Environmental Quality.

James Rudrik, Ph.D. has been appointed as the section chief of the microbiology section. Rudrik previously was the bioterrorism laboratory coordinator. Replacing Rudrik as the new bioterrorism laboratory coordinator is Valerie Reed. Reed comes to MDCH from Regional Medical Laboratories in Battle Creek.

Martha Boehme has joined the bureau as the quality assurance microbiologist working on the National Antimicrobial Resistance Project. Boehme comes to the department from Sparrow Hospital in Lansing.

Jayshree Patel of the mycobacteriology/mycology unit, has left the department to seek employment in the Michigan Department of Agriculture laboratory.

After 33 years of service at the department, Fred Platte has retired from the data acquisition and specimen handling unit. The bureau wishes him the best in his retirement.

Recent Staff Publications

Joel Blostein and Patricia Clark
Cost-effectiveness of preimmunization hepatitis B screening in high-risk adolescents, Public Health Reports, 116(2).

CDC, F.P. Downes, P. Somsel, et al.
Laboratory-acquired meningicoccal disease- United States, 2000, MMWR, 51(7).